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NEUROTYPIC AND GLIOTYPIC PROTEINS AS BIOCHEMICAL INDICATORS OF NEUROTOXICITY

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16. ABSTRACT

Exposure of the developing or mature nervous system to neurotoxic xenobiotics results in complex behavioral, physiological and morphological alterations. With few exceptions, a biochemical basis for these toxicant-induced changes has yet to be described (Damstra and Bondy, 1982). In this chapter I will briefly address the issues to be considered when developing a biochemical approach to neurotoxicity assessment and I will review the progress of work concerning one strategy: the use of neurotypic and gliotypic proteins as biochemical indicators of neurotoxicity.

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Exposure of the developing or mature nervous system to neurotoxic xenobiotics results in complex behavioral, physiological and morphological alterations. With few exceptions, a biochemical basis for these toxicant-induced changes has yet to be described (Damstra and Bondy, 1982). In this chapter I will briefly address the issues to be considered when developing a biochemical approach to neurotoxicity assessment and I will review the progress of work concerning one strategy: the use of neurotypic and gliotypic proteins as biochemical indicators of neurotoxicity.

What is neurotoxicity?

Perhaps one of the most confusing issues confronting the student of neurotoxicology is the differences of opinion with respect to what constitutes a neurotoxic response. Broadly defined neurotoxicity includes any unwanted neuroactive effect and encompasses both functional and structural alterations. A more restricted definition of neurotoxicity assumes that the common thread linking neurotoxic effects is nervous system damage initiated by an exog enous factor (Dyan, 1984). For the purposes of this review neurotoxicity will be defined as the slowly reversible or irreversible effects of toxic agents manifested by alterations in the cellular and subcellular components of the developing or mature nervous system. In this context, pharmacological effects, which by nature are short-acting, reversible and presumably non-harmful (unwanted side-effects notwithstanding) will not be considered.

What types of agents are neurotoxic?

On the basis of this operational definition of neurotoxicity one may next consider the types or classes of agents that are neurotoxic. Here, one is confronted with a dilemma of major proportions, for it is becoming increasingly apparent that an enormous chemical variety of environmental pollutants can affect nervous system structure and function (Spencer and Schaumburg, 1980). These can range from chemicals of known but widely dissimilar structure, such as pesticides and industrial chemicals, to chemical mixtures of unknown composition such as those found in toxic waste dumps. Thus, the investigator who wishes to establish a biochemical basis for neurotoxicity must face the formidable challenge of choosing neurochemical indices which are sensitive to the effects of structurally diverse, and in some cases, unknown chemical toxicants.

What are cellular targets of neuroxicity?

Unfortunately, assessing neurotoxicity is complicated not only by the variety of potential neurotoxicants (exogenous variables) but also by the innate molecular and cellular heterogeneity of the nervous system itself (endogenous variables). This inherent complexity of nervous system tissue is of concern to the neurotoxicologist because neurotoxicant-induced injury to the developing or mature organism often is manifested by alterations in the cytoarchitecture of specific neuroantomical regions (Spencer and Schaumburg, 1980). Moreover, within an affected area the response to chemical insult may encompass one or several types of neurons and glia, the major cellular elements of the nervous system. This regional and cell-type specific action of toxic agents is widely documented in the neuropathology literature and often is referred to as selective vulnerability (e.g. see

Spencer and Schaumburg, 1980; Spencer et al., 1985; and Price et al., 1986.)

Examples of selective vulnerability to neurotoxicity

To illustrate the concept of selective vulnerability, the extreme effects of three known neurotoxicants are shown in Figure 1. The first toxicant, trimethyltin Suzuki, 1980). During both prenatal and postnatal development, exposure to neurotoxicants can affect processes critical to normal nervous-system maturation, such as neurogenesis, gliogenesis, myelinogenesis and synaptogenesis. These cell-type-specific events can also be characterized by measuring neurotypic and gliotypic proteins. For example, the ontogeny of the nerve terminal protein, synapsin I (Lohmann et al., 1978) and the myelin-specific protein, myelin basic protein (Banik and Smith, 1977), coincide with two critical processes in postnatal maturation: synapse formation and myelination, respectively. Thus, the ontogeny of cellular and subcellular elements associated with specific developmental processes, as well as neurotoxicant-induced changes in these events, can be detected and quantified by measuring the appropriately selected proteins.

Because samples of nervous system tissue are required for most biochemical analyses, neurotoxicity normally is assessed in experimental animals. It is conceivable, however, that by measuring specific proteins in accessible biological fluids, such as cerebrospinal fluid or blood, it may be possible to detect and quantify neurotoxicity in the exposed human population. One example of a protein that has been used to diagnose damage to a specific brain component is myelin basic protein; it has been found in the CSF of patients suffering from the demyelinating disease, multiple sclerosis (Cohen et al., 1980). Due to proteolytic degredation, neurotypic and gliotypic proteins released into CSF are unlikely to be found in blood in their intact form. Antigenic determinents unique to specific proteins, however, may be detected by monoclonal antibodies recognizing these specific sites on proteolytic fragments of the native protein. If this turns out to be the case, it may be possible to develop a blood test for neurotoxicity based on the presence of unique protein determinants in the serum of individuals exposed to neurotoxicants.

In summary, it appears likely that neurotypic and gliotypic proteins represent versatile biochemical endpoints for detecting and characterizing neurotoxicity. Some of the advantages inherent to this approach are as follows:

1. Prior knowledge of the nature of the toxicant(s) being evaluated is not required. By measuring neurotypic and gliotypic proteins, one is not restricted to analysis of specific brain regions, or limited to specific neurotransmitter systems or tied to specific functional endpoints.

In theory, this permits the effects of agents as diverse as unknown mixtures, such as hazardous wastes, or physical hazards, such as radiation, to be detected and characterized throughout the central and peripheral nervous systems.

2. Neurotypic and gliotypic proteins appear to be as numerous and diverse as the cell types they represent. Thus, when applied to neurotoxicology,

one is not restricted to measuring a select number or type of protein. Instead, one can take advantage of current advances in neuroscience that continue to reveal the extensive cellular heterogeneity of the nervous system based on the discovery of novel neurotypic and gliotypic proteins (e.g. see De Blas et al., 1984; Nestler and Greengard, 1984; Hockfield and McKay, 1985)

3. Because neurotypic and gliotypic proteins are intimately linked to nervous system cytoarchitecture, measurements of these proteins make it possible to establish an integrated molecular and cellular basis of neurotoxicity. For example, radioimmunoassays (RIAs) of specific proteins can be combined with immunohistochemical localization of these proteins using the same antibodies. In effect, this allows for a qualitative localization of the affected region to be combined with a quantitative analysis of the proteins associated with the affected cell type. By documenting the effects of toxicant exposure in this manner, a cellular and molecular basis for interpreting changes in specific aspects of whole animal function (behavioral or physiological) may ultimately be established.

Effects of known neurotoxicants on neurotypic and gliotypic proteins. In order to validate the use of neurotypic and gliotypic proteins as biochemical indicators of neurotoxicity, we have administered known neurotoxicants to experimental animals to assess the effects of these agents on several previously characterized proteins. One neurotoxic chemical employed in our studies is TMT (see Fig. 1). As noted above, TMT preferentially damages the hippocampus (Brown et al., 1979) where it causes consistent dose-related decrements in pyramidal neurons. By employing TMT as a denervation tool to alter the morphology of the hippocampus, we have been able to demonstrate accompanying changes in neuron-specific proteins (O'Callaghan and Miller, 1984; Miller and O'Callaghan, 1984; Brock and O'Callaghan, submitted). The effects of TMT on one of these proteins, synapsin I, are shown in Fig 2. Three weeks following acute administration of TMT to the rat, large dose-related decreases in synapsin I were observed in hippocampus. Consistent with the region-selective effects of TMT, decrements in synapsin I were not observed in frontal cortex, a brain opathol. Exp . Neurol 18: 165-189.

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Figure Legends

Figure 1. Examples of selective vulnerability to neurotoxicity. Micrographs were prepared from cresyl violet stained sagittal sections of rat hippocampus (upper panels), cerebellum (middle panels) and whole brain (lower panels). The neurotoxic agents were trimethyltin (TMT), bilirubin (the hyperbilirubinemic, jj, Gunn rat was used as a model) and cadmium (Cd++). For a description of subjects and dosing regimens, see O'Callaghan and Miller (1984) (TMT), O'Callaghan and Miller (1985) (bilirubin) and O'Callaghan and Miller (19 86), (Cd++).

Figure 2. Effects of TMT on synapsin I in rat hippocampus and frontal cortex. Subjects were killed 21 days after treatment. Each value (N = 4-5) represents the mean relative specific activity + SEM expressed on a concentration (per mg total protein) basis. Values obtained for hippocampus-saline (0 mg/kg) were used to calculate a mean relative specific activity = 1.0 (100%). * Significantly different from corresponding saline control, $P < 0.01$.

Figure 3. Effects of TMT on glial fibrillary acidic protein in rat hippocampus and frontal cortex. Subjects were killed 21 days after treatment. Each value (N = 4-5) represents the mean relative specific activity + SEM expressed on a concentration (per mg total protein) basis. Values obtained for hippocampus saline (0 mg/kg) were used to calculate a mean relative specific activity = 1.0 (100%). * Significantly different from corresponding.

Figure 4. Astrocytic response to TMT-induced damage of the rat hippocampus (areas CA3c-CA4) as revealed by immunohistochemistry of GFAP. Subjects were killed 21 days after administration of 8.0 mg/kg of TMT.

Figure 5. Effects of acute administration of triethyltin (TET) on glial fibrillary acidic protein (GFAP), p38, neurofilament 200 (NF 200) and myelin basic protein (MBP) in the developing rat. TET (0.0, 3.0 and 6.0 mg/kg) was administered on postnatal day 5 and neurotypic and gliotypic proteins were assayed on postnatal days 13, 22, 40 and 66. Each value (N = 8) represe nts the mean relative specific activity + SEM expressed on a concentration (per mg total protein) basis. For each protein, values obtained for forebrain saline controls (data not shown) were used to calculate a mean relative specific activity = 1.0 (100%).

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Table I

Examples of neurotypic and gliotypic proteins suitable for use as biochemical indicators of neurotoxicity

Protein	Regional Distribution	Cellular Distribution	Reference
synapsin 1	all neurons in CNS & PNS	synaptic vesicles (cytoplasmic surface)	Nestler and Greengard, 1984
protein III	all neurons in CNS & PNS & adrenal chromaffin cells	synaptic vesicles chromaffin granules	Nestler and Greengard, 1984
p38	all neurons in CNS & PNS; tissues with secretory vesicles	synaptic veiscles (integral membrane protein); secretory vesicles	Jahn et al., 1985
cGMP-dependent protein kinase	cerebellar Purkinje cells	cytosol	De Camilli et al., 1984a
G-substrate	cerebellar Purkinje cells	cytosol	Detre et al., 1984
PCPP-260	cerebellar Purkinje cells	membranes	Walaas et al., 1986
CaM kinase II	CNA neurons	soma, dendrites postsynaptic density	Ouimet et al., 1984
neurofilament p68, 160, 200	all neurons	axon intermediate filaments	Shaw et al., 1981
DARPP-32	dopaminoceptive neurons that possess D-1 receptors	cytosol	Nestler and Greengard, 1984
PCM	CNS neurons	cytosol	Billingsley and Balaban, 1985
MAP-2	CNS neurons	microtubules	De Camilli et al., 1984b
NSE	CNS neurons, peripheral neuroendocrine cells	cytosol	Kirino et al., 1983
B-50	CNS neurons	presynaptic membranes	Gispen et al., 1985
GFAP	astrocytes	intermediate filaments	Eng, 1985
myelin basic protein	oligodenrocytes	myelin	Schwob et al., 1985
proteoliped protein	oligodenrocytes	myelin	Schwob et al., 1985
myelin associated glycoprotein	oligodenrocytes	myelin	Sternberger et al., 1979

Abbreviations: PCPP-260, Purkinje cell-specific phosphoprotein, M_r 260,000; CaM, calcium/cal modulin; DARPP-32, dopamin, cAMP-regulated phophoprotein, M_r 32,000; PCM, protein-O-carboxy-methyltransferase; MAP, microtubule associated protein; GFAP, glial fibrillary acidic protei

TABLE 2

Cerebellar Hypoplasia in the Gunn Rat is Accompanied by Changes in Neurotypic and Gliotypic Proteins

Cerebellum Weight (g)	Relative Specific Activity				
	G-substrate	PCPP-260	Synapsin I	GFAP	MBP
0.30 ± .01	1.00 ± .09	1.00 ± .03	1.00 ± .11	1.00 ± .06	1.00 ± .05
0.15 ± .02**	0.36 ± .09**	0.40 ± .10**	0.63 ± .06*	3.81 ± .61*	1.23 ± .03**

Each value represents the mean relative specific activity ± S.E.M. expressed on concentration (per milligram of protein) basis.

* Significantly different from Jj controls, $P < 0.01$.

** Significantly different from Jj controls, $P < 0.001$

Figure 1

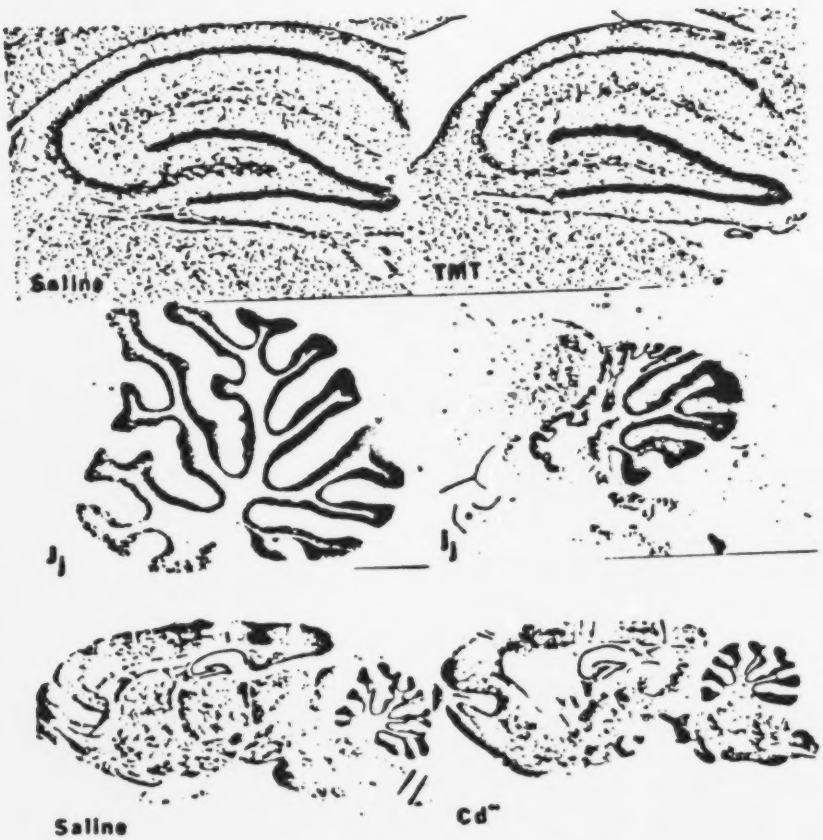


Figure 2

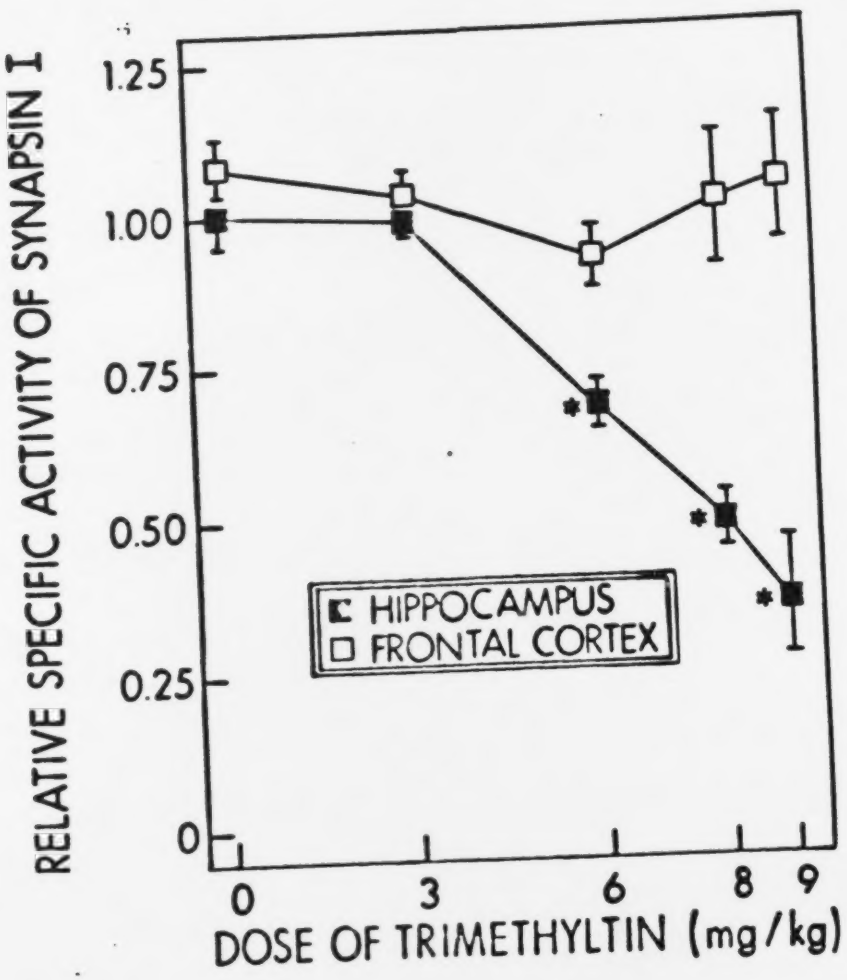
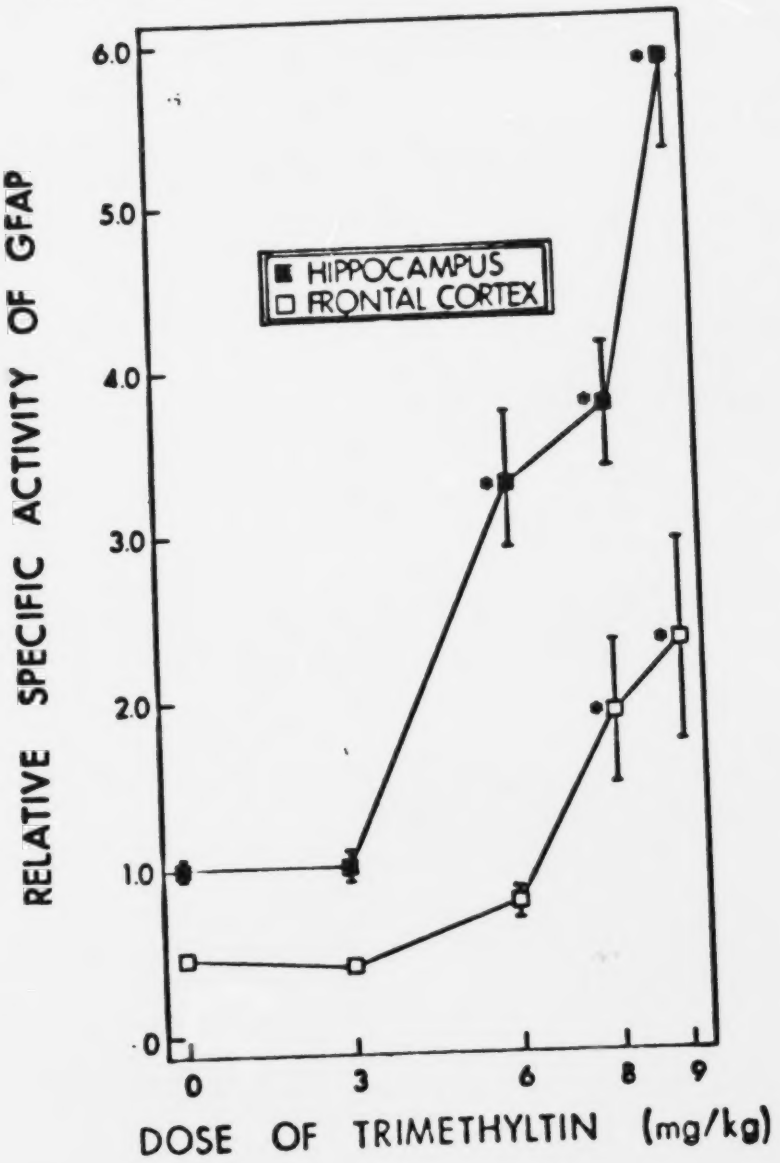

RELATIVE SPECIFIC ACTIVITY OF SYNAPSIN I
1.25
1.00
0.75
0.50
0.25
0
HIPPOCAMPUS
FRONTAL CORTEX
0
3
6
8
9
DOSE OF TRIMETHYLTIN (mg/kg)

Figure 3


RELATIVE SPECIFIC ACTIVITY OF GFAP
6.0
5.0
4.0
3.0
2.0
1.0
0
HIPPOCAMPUS
FRONTAL CORTEX
0
3
6
8
9
DOSE OF TRIMETHYLTIN (mg/kg)

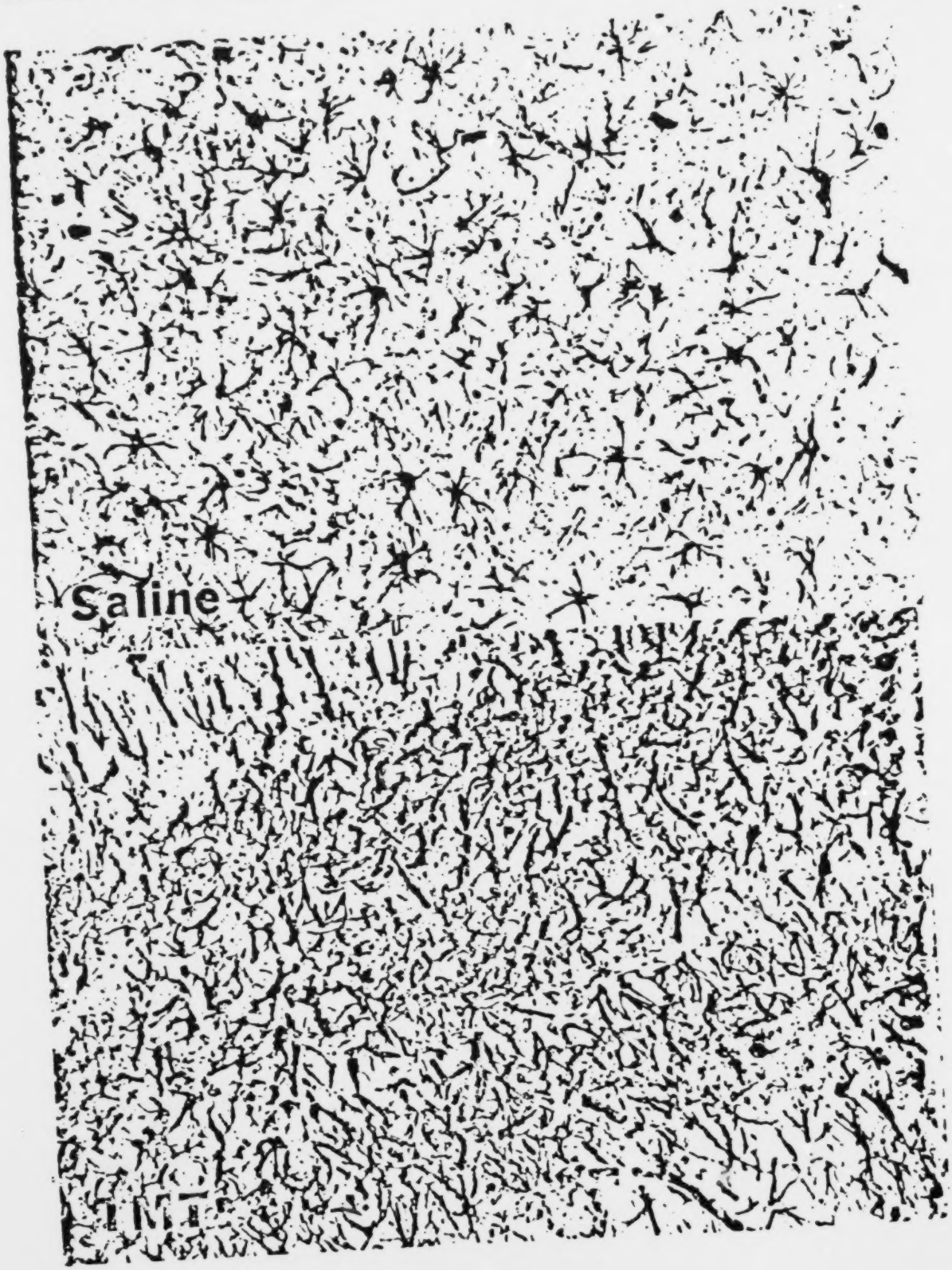
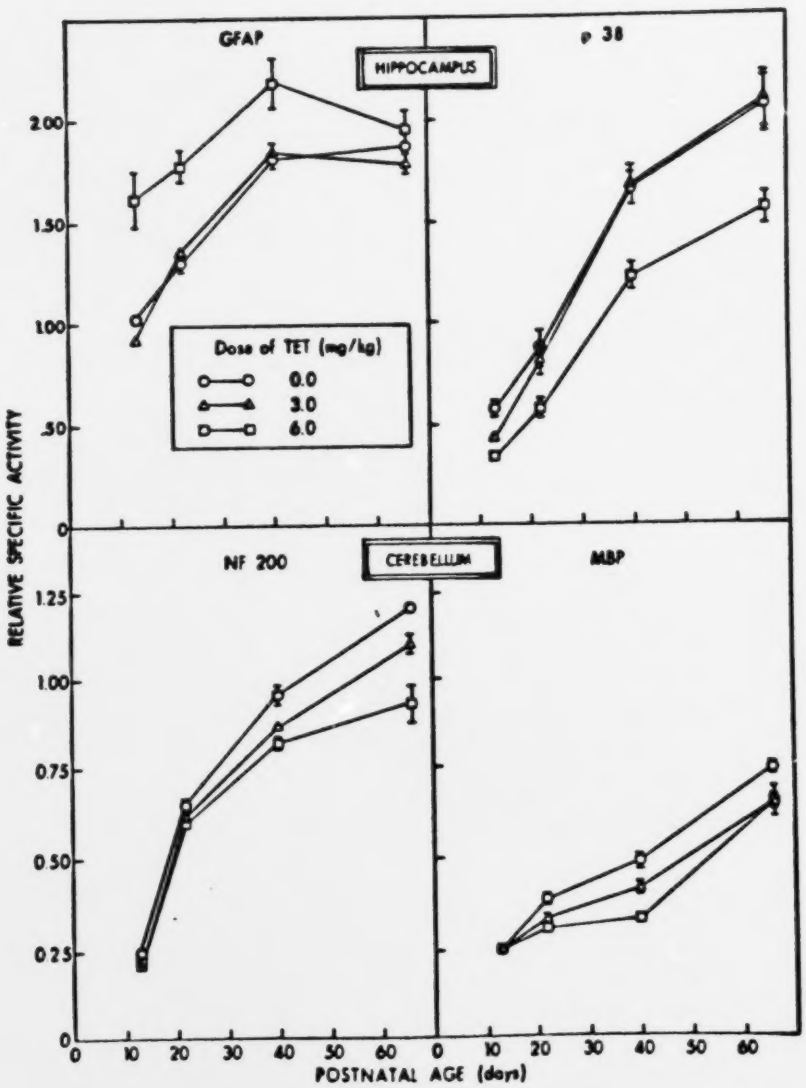


Figure 4

Figure 5


GFAP
HIPPOCAMPUS
p 38
Dose of TET (mg/kg)
0.0
3.0
6.0
NF 200
CEREBELLUM
MBP
RELATIVE SPECIFIC ACTIVITY
POSTNATAL AGE (days)